
**Cereals, cereals-based products
and animal feeding stuffs —
Determination of crude fat and total
fat content by the Randall extraction
method**

*Céréales, produits céréaliers et aliments des animaux —
Détermination de la teneur en matières grasses brutes et en matières
grasses totales par la méthode d'extraction de Randall*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2. www.iso.org/directives

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This second edition of ISO 11085 cancels and replaces the first edition (ISO 11085:2008), which has been technically revised.

Cereals, cereals-based products and animal feeding stuffs — Determination of crude fat and total fat content by the Randall extraction method

1 Scope

This International Standard specifies procedures for the determination of the fat content of cereals, cereal-based products, and animal feeding stuffs. These procedures are not applicable to oilseeds and oleaginous fruits.

The choice of procedure to be used depends on the nature and composition of the material analysed and the reason for carrying out the analysis.

Procedure A is a method for the determination of directly extractable crude fats, applicable to all materials, except those included within the scope of procedure B.

Procedure B is a method for the determination of total fats, applicable to all materials from which the oils and fats cannot be completely extracted without prior hydrolysis.

NOTE Most cereals, as well as feeds of animal origin, yeasts, potato protein, compound feeds with milk products, glutens, and products subjected to processes such as extrusion, flaking, and heating, yield significantly higher total fat contents when tested by procedure B than by procedure A. See [Annex B](#).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

crude fat content

mass fraction of substances extracted from the sample by the specified procedure A

Note 1 to entry: The crude fat content is expressed as a percentage mass fraction.

3.2

total fat content

mass fraction of substances extracted from the sample by the specified procedure B

Note 1 to entry: The total fat content is expressed as a percentage mass fraction.

4 Principle

Fat is extracted using light petroleum as a solvent and the Randall modification of the Soxhlet method. The test portion is submerged in boiling solvent prior to rinsing in cold solvent, reducing the time needed for extraction. The solvent dissolves fats, oils, pigments, and other soluble substances. After

extraction, the solvent is evaporated and recovered by condensation. The resulting fat residue is determined gravimetrically after drying.

For total fat determination, the sample is treated under heating with hydrochloric acid. Hydrolysis makes chemically or mechanically bound fats accessible to solvent extraction. The mixture is cooled and filtered. The residue is washed and dried and submitted to the above extraction procedure.

For total fat determinations of samples with a "high" fat content (i.e. at least 150 g/kg), a preliminary extraction is performed before applying procedure B.

5 Reagents

Use only reagents of recognized analytical grade.

5.1 Water, complying with the requirements of at least grade 3 of ISO 3696.

5.2 Light petroleum (petroleum ether), consisting mainly of hydrocarbons with six carbon atoms, boiling range 30 °C to 60 °C. The bromine value shall be less than one. The evaporation residue shall be less than 20 mg/l.

5.3 Glass beads, of diameter 5 mm to 6 mm or **silicon carbide chips**.

5.4 Hydrochloric acid, $c(\text{HCl}) = 3 \text{ mol/l}$.

5.5 Filtration aid, e.g. diatomaceous earth, boiled for 30 min in hydrochloric acid, $c(\text{HCl}) = 6 \text{ mol/l}$, washed with water ([5.1](#)) until acid-free, then dried at 130 °C.

5.6 Acetone.

5.7 Cotton wool, defatted.

5.8 Fat-free filter paper.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Solvent extraction system, consisting of a 2-stage Randall extraction process unit enabling solvent recovery, fitted with fluoroelastomer or polytetrafluoroethylene seals compatible with petroleum ether.

6.2 Hydrolysis apparatus I, multiple position unit enabling boiling with acid, compatible with the solvent extraction system ([6.1](#)), used for hydrolysis according to [8.4.1](#).

6.3 Hydrolysis apparatus II, consisting of either a beaker of capacity 400 ml and, as a cover, a watch glass of appropriate diameter, or a conical flask of capacity 300 ml with a reflux condenser, used for hydrolysis according to [8.4.2](#).

6.4 Drying oven, capable of being maintained at $(103 \pm 2) \text{ °C}$.

6.5 Microwave oven, with defrost setting.

6.6 Desiccator, containing an efficient desiccant.

6.7 Filter cartridge, adapted to the hydrolysis apparatus used.

6.8 Extraction thimbles, of cellulose, free from petroleum ether-extractable products, and **stand** to hold thimbles.

6.9 Extraction cups, of aluminium or glass, compatible with the solvent extraction system (6.1).

6.10 Glass thimbles for hydrolysis.

6.11 Analytical balance, enabling weighing at 10^{-2} mg accuracy.

6.12 Mill or grinder, fitted with a 1 mm screen or for samples with a fat mass fraction of between 15 % and 20 %, a **water-cooled knife mill**.

6.13 Büchner funnel.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport and storage.

Sampling is not part of the method specified in this International Standard. Recommended sampling methods are given in ISO 24333.

8 Procedure

8.1 Preparation of the test sample

Grind (6.12) laboratory samples to a particle size < 1 mm.

For animal feeding stuff, prepare the test sample as specified in ISO 6498.

8.2 Test portion

The test portion consists of 1 g to 5 g, m_1 , of the ground test sample weighed to the nearest 1 mg.

If the fat content of the test sample is higher than 150 g/kg, start the procedure with 8.3 for total fat determination and continue with 8.4 and 8.5.

In all other cases, start the procedure with 8.4 for total fat determination (procedure B) and with 8.5 for crude fat determination (procedure A).

8.3 Preliminary extraction

8.3.1 Comply with the manufacturer's instructions for the operation of the solvent extraction system (6.1).

8.3.2 Add 5 to 10 glass beads (5.3) and place the extraction cups (6.9) in the drying oven (6.4) for at least 30 min at $103 \text{ °C} \pm 2 \text{ °C}$. Transfer the extraction cups to a desiccator (6.6) and cool to room temperature. Weigh the extraction cups and record their mass, m_2 , to the nearest 0,1 mg.

8.3.3 Weigh the test portion into a glass thimble (6.10), if using hydrolysis apparatus I (6.2) or into an extraction thimble (6.8) if using hydrolysis apparatus II (6.3).

If recommended by the manufacturer, add filtration aid (5.5).

8.3.4 Set the temperature to achieve a reflux of light petroleum (5.2) that is 3 drops/s to 5 drops/s (about 10 ml/min). Preheat the instrument and make sure the cooling water for the reflux condenser is turned on. With cooling water at approximately 15 °C, the flow should be adjusted to 2 l/min to prevent solvent evaporation from the condensers.

8.3.5 Place thimbles containing test portions in the extraction columns. Place the cups under the extraction columns and secure in place. Add 40 ml to 60 ml, following the manufacturer's instructions, of light petroleum to each extraction cup. Make sure that the cups are matched to their corresponding thimble.

8.3.6 Rinse with light petroleum (5.2) for 20 min and recover the solvent for 10 min.

8.3.7 Remove the extraction cups from the extractor and place in an operating fume hood. Let cups remain in the hood until all traces of solvent are gone.

8.3.8 Dry the cups at 103 °C ± 2 °C in the drying oven (6.4) for 30 min. Excessive drying might oxidize the fat and give high results. Cool in a desiccator (6.6) to room temperature and weigh to the nearest 0,1 mg, m_3 .

Proceed in accordance with 8.4.

8.4 Hydrolysis

8.4.1 General

Follow either 8.4.1 or 8.4.2.

8.4.2 Hydrolysis with apparatus I (6.2)

For the hydrolysis, comply with the manufacturer's instructions.

Transfer the thimbles (6.10) containing the pre-extracted test portion or, if no pre-extraction has been used, weigh the test portion, m_1 , into a thimble (6.10) for hydrolysis apparatus I (6.2). Add filtration aid (5.5), if needed, and 130 ml HCl (5.4) to each test portion, and bring to the boil. Maintain the liquid at boiling point for 1 h. Filter on the cartridge (6.7) and wash the residue with warm (60 °C) water (5.1) until acid free. Clean all surfaces where fat can stick with cotton wool (5.7) soaked in acetone (5.6). Add the cotton wool used for cleaning to the residue in the thimble (6.10) and dry residue to constant mass, e.g. by heating in a microwave oven (6.5) at defrost setting for 1 h. Ensure that all acetone has evaporated before drying.

8.4.3 Hydrolysis with apparatus II (6.3)

Transfer the pre-extracted test portion or weigh the test portion, m_1 , to the beaker or conical flask (6.3). Add 100 ml of hydrochloric acid (5.4) and silicon carbide chips (5.3). Cover the beaker with a watch glass or fit the conical flask with a reflux condenser. Bring the mixture to a gentle boil over a flame or a hot plate and maintain it at boiling point for 1 h. Swirl every 10 min to prevent the product sticking to the sides of the container.

Cool to ambient temperature and add a quantity of filtration aid (5.5) sufficient to prevent any loss of fat during the filtration. Filter through a moistened, fat-free double filter paper (5.8) in a Büchner funnel (6.13) with suction. Wash the residue with cold water (5.1) until a neutral filtrate is obtained. Clean all surfaces where fat can stick with cotton wool (5.7) soaked in acetone. Add the cotton wool

used for cleaning to the residue in the filter and dry to constant mass, e.g. by heating in a microwave oven (6.5) at defrost setting for 1 h. Ensure that all acetone has evaporated before drying.

CAUTION — If oil or fat appears on the surface of the filtrate, erroneous results might be obtained. A possible solution is to repeat the procedure using either a smaller test portion or, preferably, the pre-extraction procedure (8.3).

After drying, take out the filter (5.8) containing the residue and the defatted cotton wool, place them in an extraction thimble (6.8) and cover with a wad of cotton wool (5.7).

8.5 Extraction

8.5.1 For the extraction, comply with the manufacturer's instructions for the operation of the extractor.

8.5.2 Add 5 to 10 glass beads (5.3) and dry the extraction cups (6.9) in the drying oven (6.4) for 30 min or until constant mass at $103\text{ °C} \pm 2\text{ °C}$. Transfer to a desiccator (6.6) and cool to room temperature. Weigh the extraction cups and record the mass, m_4 , to the nearest 0,1 mg.

8.5.3 Set the temperature to achieve a reflux of solvent that is 3 drops/s to 5 drops/s (about 10 ml/min). Preheat the extraction unit (6.1) and make sure the cooling water for the refluxing condensers is turned on. With cooling water at approximately 15 °C , the flow should be adjusted to 2 l/min to prevent solvent evaporation from the condensers.

8.5.4 Attach thimbles containing test portions (8.2) or test portions from hydrolysis (8.4) to the extraction columns. Place the cups under the extraction columns and secure in place. Make sure that the cups are matched to their corresponding thimble.

8.5.5 Add a volume of light petroleum (5.2) to each extraction cup that is sufficient to cover the test portion when the thimbles are in position for boiling.

8.5.6 Maintain the light petroleum (5.2) at boiling point for 20 min, rinse for 40 min, and recover the solvent for 10 min.

8.5.7 Remove the extraction cups from the extractor and place them under an operating fume hood. Leave the cups under the hood until all traces of solvent have disappeared.

8.5.8 Dry the cups at $103\text{ °C} \pm 2\text{ °C}$ in the drying oven (6.4) for 2 h, sufficient time to eliminate water. Excessive drying may oxidize the fat and give high results. Cool in a desiccator to room temperature, weigh, and record the mass, m_5 , to the nearest 0,1 mg.

9 Calculation and expression of results

9.1 Determination with preliminary extraction

Calculate the fat content of the test sample, w_1 , as a percentage mass fraction, using Formula (1):

$$w_1 = \left[\left(\frac{m_3 - m_2}{m_1} \right) + \left(\frac{m_5 - m_4}{m_1} \right) \right] \times 100 \quad (1)$$

where

m_1 is the mass, in grams, of the test portion (8.2);

m_2 is the mass, in grams, of the extraction cup with glass beads used in 8.3;

m_3 is the mass, in grams, of the extraction cup with glass beads and the dried light petroleum extract residue obtained in 8.3;

m_4 is the mass, in grams, of the extraction cup with glass beads used in 8.5;

m_5 is the mass, in grams, of the extraction cup with glass beads and the dried light petroleum extract residue obtained in 8.5.

Express the result to the nearest 0,1 %.

9.2 Determination without preliminary extraction

Calculate the fat content of the test sample, w_2 , as a percentage mass fraction, using Formula (2):

$$w_2 = \left(\frac{m_5 - m_4}{m_1} \right) \times 100 \quad (2)$$

Express the result to the nearest 0,1 %.

10 Precision

10.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in [Annex A](#). The values derived from this interlaboratory test cannot be applied to other concentration ranges and matrices than those given.

10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time will, in no more than 5 % of cases, be greater than the following repeatability limits.

For procedure A, for products whose fat content is between 0,48 g/100 g and 25,77 g/100 g (see [Table A.1](#) and [Figure A.1](#)), $r = 0,25$.

For procedure B, for products whose fat content is between 1,07 g/100 g and 27,08 g/100 g (see [Table A.2](#) and [Figure A.2](#)), $r = 0,35$.

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will, in no more than 5 % of cases, be greater than the following reproducibility limits.

For procedure A, for products whose fat content is between 0,48 g/100 g and 25,77 g/100 g (see [Table A.1](#) and [Figure A.1](#)), $R = 0,63$.

For procedure B, for products whose fat content is between 1,07 g/100 g and 27,08 g/100 g (see [Table A.2](#) and [Figure A.2](#)), $R = 1,10$.

10.4 Critical difference

10.4.1 General

When the difference between two averaged values obtained from two test results under repeatability conditions is to be assessed, the repeatability limit cannot be used. Use the critical difference.

10.4.2 Comparison of two groups of measurements in one laboratory

The critical difference between two averaged values obtained from two test results under repeatability conditions, CD_r , is given by Formula (3):

$$CD_r = 2,8 s_r \sqrt{\frac{1}{2n_1} + \frac{1}{2n_2}} = 2,8 s_r \sqrt{\frac{1}{2}} = 1,98 s_r \quad (3)$$

where

s_r is the standard deviation of repeatability;

n_1, n_2 are the numbers of test results corresponding to each of the averaged values (here, $n_1 = n_2 = 2$).

The absolute difference between two averaged values obtained from two test results under repeatability conditions, will, in no more than 5 % of cases be greater than the following critical differences.

For procedure A, for products whose fat content is between 0,48 g/100 g and 25,77 g/100 g, $CD_r = 0,42$.

For procedure B, for products whose fat content is between 1,07 g/100 g and 27,08 g/100 g, $CD_r = 0,78$.

10.4.3 Comparison of two groups of measurements in two laboratories

The critical difference between two averaged values obtained in two different laboratories from two test results under repeatability conditions, CD_R , is given by Formula (4):

$$CD_R = 2,8 \sqrt{s_R^2 - s_r^2 \left(1 - \frac{1}{2n_1} - \frac{1}{2n_2}\right)} = 2,8 \sqrt{s_R^2 - 0,5 s_r^2} \quad (4)$$

where

s_R is the standard deviation of reproducibility.

The absolute difference between two averaged values obtained in two different laboratories from two test results under repeatability conditions will, in no more than 5 % of the cases be greater than the following critical differences.

For procedure A, for products whose fat content is between 0,48 g/100 g and 25,77 g/100 g, $CD_R = 0,57$.

For procedure B, for products whose fat content is between 1,07 g/100 g and 27,08 g/100 g, $CD_R = 1,07$.

10.5 Measurement uncertainty

Measurement uncertainty is a parameter characterizing the dispersion of values that can reasonably be attributed to the result. This uncertainty is established through the statistical distribution of results given by the interlaboratory test and characterized by the experimental standard deviation.

In this International Standard, the uncertainty, u , is equal to plus or minus twice the reproducibility standard deviation.

For procedure A, for products whose fat content is between 0,48 g/100 g and 25,77 g/100 g, $u = \pm 0,40$.

For procedure B, for products whose fat content is between 1,07 g/100 g and 27,08 g/100 g, $u = \pm 0,80$.

11 Test report

The test report shall contain at least the following details:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, with reference to this International Standard, i.e. ISO 11085;
- d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- e) whether preliminary extraction ([8.3](#)) was used;
- f) the test result obtained, or the two test results obtained, if the repeatability has been checked.

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Annex A (informative)

Results of an interlaboratory test

An interlaboratory test organized by FOSS AB (Sweden) in 2005, involving 15 laboratories in eight countries, was carried out on 11 samples representing different cereals, cereal products, and animal feeding stuffs.

Two laboratories had to be excluded due to non-compliance with the method specified. One laboratory did not submit data for procedure B.

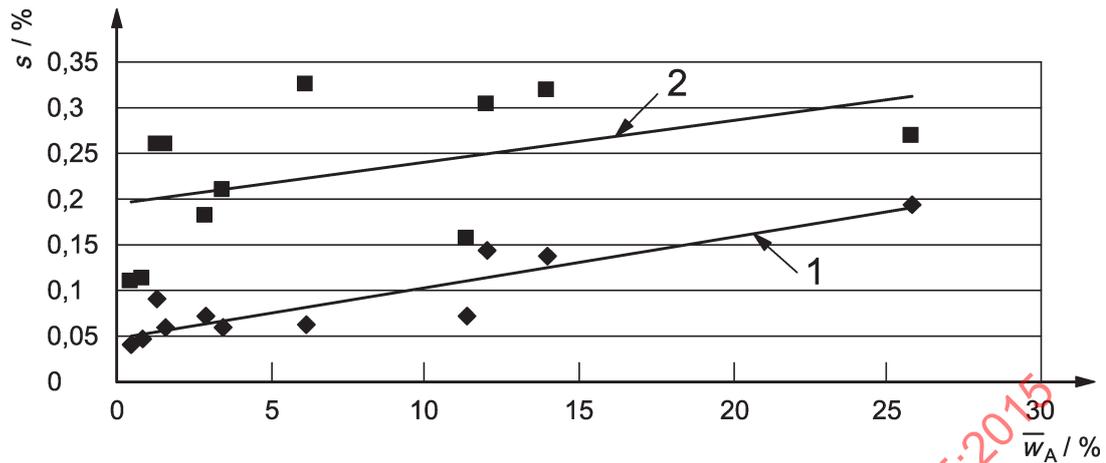
The results of the remaining 13 laboratories for procedure A and 12 laboratories for procedure B were subjected to statistical analysis in accordance with ISO 5725-2^[2] to give the precision data shown in [Table A.1](#) and [Table A.2](#).

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Table A.1 — Results of statistical analysis for crude fat (procedure A)

Sample (No.)	Rice, par-boiled (1)	Whole wheat kernels (2)	Rye flour (3)	Sorghum kernels (4)	Couscous (durum wheat) (5)	Flour mix (multicorn) (6)	Croutons (7)	Cornbread (8)	Cattle feed (9)	Chicken feed (10)	Pig feed (11)
No. laboratories	13	13	13	13	13	13	13	13	13	13	13
No. laboratories retained after elimination of outliers	11	13	13	13	13	12	12	11	11	13	13
Mean of crude fat content (procedure A), g/100 g	0,481	1,621	1,316	3,412	0,842	11,362	13,969	25,773	11,943	6,19	2,928
Repeatability standard deviation, s_r , g/100 g	0,041	0,058	0,092	0,058	0,046	0,070	0,137	0,193	0,145	0,063	0,073
Coefficient of variation of repeatability, $C_{V,r}$, %	8,5	3,5	6,9	1,7	5,5	0,6	1,0	0,8	1,2	1,0	2,5
Repeatability limit, $r = 2,8 \times s_r$, g/100 g	0,114	0,161	0,257	0,164	0,129	0,196	0,384	0,542	0,407	0,177	0,203
Reproducibility standard deviation, s_R , g/100 g	0,109	0,258	0,259	0,210	0,111	0,157	0,318	0,270	0,303	0,324	0,182
Coefficient of variation of reproducibility, $C_{V,R}$, %	22,6	15,8	19,5	6,2	13,1	1,4	2,3	1,0	2,5	5,2	6,2
Reproducibility limit, $R = 2,8 \times s_R$, g/100 g	0,304	0,723	0,725	0,588	0,310	0,440	0,891	0,757	0,849	0,906	0,509

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**Key**

s standard deviation

\bar{w}_A mean crude fat content (procedure A)

1 standard deviation of repeatability, $s_r = 0,005\ 5 \bar{w}_A + 0,048\ 5$; $R^2 = 0,797\ 8$

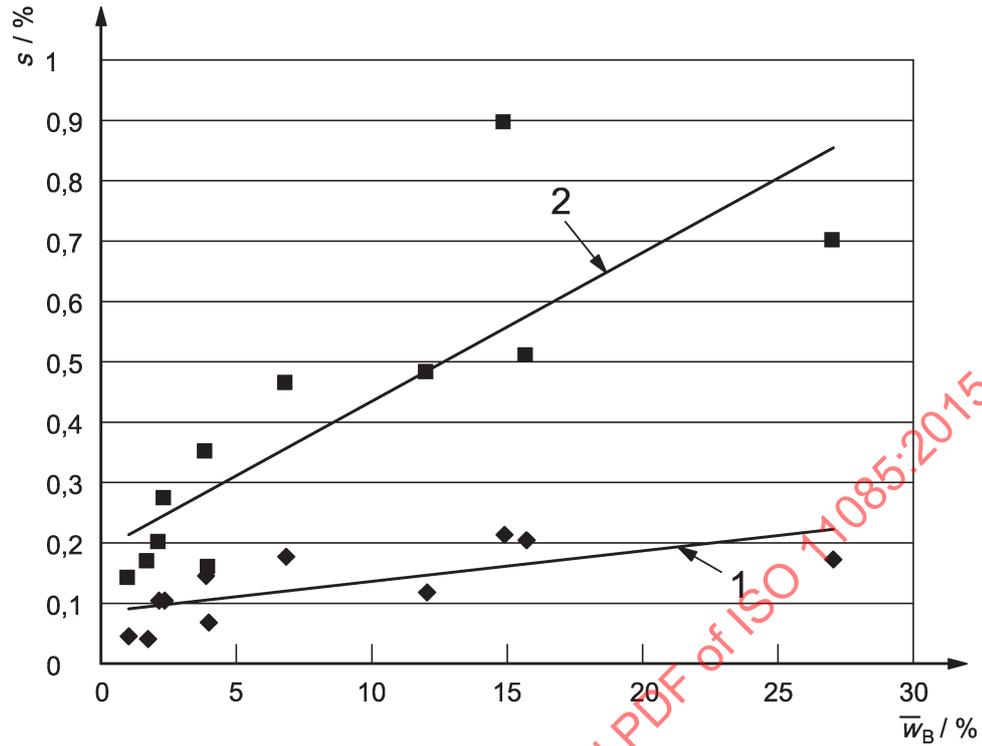
2 standard deviation of reproducibility, $s_R = 0,004\ 6 \bar{w}_A + 0,194\ 2$; $R^2 = 0,207\ 5$

Figure A.1 — Standard deviations of repeatability, s_r , and reproducibility, s_R , as functions of the crude fat content (procedure A), \bar{w}_A

Table A.2 — Results of statistical analysis according to ISO 5725-2 for total fat (procedure B)

Sample (No.)	Rice, par-boiled (1)	Whole wheat kernels (2)	Rye flour (3)	Sorghum kernels (4)	Couscous (durum wheat) (5)	Flour mix (multicorn) (6)	CROUTONS (7)	Cornbread (8)	Cattle feed (9)	Chicken feed (10)	Pig feed (11)
No. of laboratories	12	12	12	12	12	12	12	12	12	12	12
No. of laboratories retained after elimination of outliers	12	12	12	10	12	12	9	11	12	12	11
Mean of total fat content (procedure B), g/100 g	1,066	2,366	1,780	4,003	2,193	12,035	15,751	27,080	14,872	6,813	3,883
Repeatability standard deviation, s_r , g/100 g	0,045	0,105	0,039	0,069	0,103	0,118	0,203	0,170	0,215	0,178	0,146
Coefficient of variation of repeatability, $C_{V,r}$, %	4,2	4,4	2,2	1,7	4,7	1,0	1,3	0,6	1,4	2,6	3,8
Repeatability limit, $r = 2,8 \times s_r$, g/100 g	0,125	0,293	0,109	0,193	0,288	0,330	0,567	0,476	0,601	0,498	0,409
Reproducibility standard deviation, s_R , g/100 g	0,143	0,271	0,169	0,158	0,199	0,480	0,511	0,698	0,896	0,463	0,351
Coefficient of variation of reproducibility, $C_{V,R}$, %	13,4	11,5	9,5	3,9	9,1	4,0	3,2	2,6	6,0	6,8	9,0
Reproducibility limit, $R = 2,8 \times s_R$, g/100 g	0,401	0,759	0,475	0,442	0,557	1,344	1,431	1,954	2,509	1,296	0,982

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Key

s standard deviation

\bar{w}_B mean total fat content (procedure B)

1 standard deviation of repeatability, $s_r = 0,005 2 \bar{w}_B + 0,083 4$; $R^2 = 0,474$

2 standard deviation of reproducibility, $s_R = 0,024 5 \bar{w}_B + 0,189 6$; $R^2 = 0,681$

Figure A.2 — Standard deviations of repeatability, s_r , and reproducibility, s_R , as functions of the mean total fat content (procedure B), \bar{w}_B